Staphylococcal Antitoxin Values in the Sera of Permanent Residents and Visitors in Thailand

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Staphylococcal food poisoning presented a differential diagnostic problem in some instances during the cholera epidemic which afflicted Thailand in 1958. Approximately 20 per cent of the food samples collected in some open markets in Thailand harboured enterotoxic staphylococci. Bacteriologically proven and clinically manifest staphylococcal food poisoning, however, was not frequent in the Thai population, while foreign visitors often suffered digestive disturbances with symptoms of staphylo-enterotoxicosis. It was assumed that this phenomenon might be due to a greater resistance of the local inhabitants against staphylococci induced by constant exposure to these organisms. It was further assumed that such resistance could be reflected by higher anti-staphylococcal antibody titres in the blood. Hence a serological investigation was carried out in Thais and in recent arrivals to Thailand.

The main part of this study was performed at the SEATO Cholera Research Laboratory in Bangkok, Thailand, the other at the Walter Reed Army Institute of Research in Washington, D.C. A preliminary report was published earlier (Felsenfeld 1959).

Materials and Methods

The sera were collected between 1958 and 1960. They came from Thai patients afflicted with cholera, other enteric infections, trachoma and from healthy enlisted personnel of the Royal Thai Army. Blood samples were collected from recently arrived Americans who reported to the Dispensary of the United States Embassy during the same period. Only such Americans were selected for this study who had been in Thailand less than 10 days. Children were not included in this sampling.

The sera were kept at −20° C. without a preservative.

The method of Kienitz and Kümmel (1960) was used for the determination of the serum anti-α toxin, using a slight modification. The buffer in which the hemolysin tests were performed consisted of 8·5 Gm sodium chloride, 0·80 Gm crystalline disodium phosphate with 12 mol. crystal water and 0·68 Gm anhydrous monopotassium phosphate in 1·0 litre distilled water of pH 7·4. Twofold dilutions of the inactivated sera were prepared in this buffer and to each 1 ml. of these dilutions 0·01 units of the α toxin in 0·5 ml. buffer were added. After incubation for 30 minutes at room temperature, 1 ml. of a 1 per cent suspension of sheep red blood cells were added. After further incubation for 1 hour at 37°C. in the water bath the mixtures were gently shaken and kept 1 hour at room temperature before reading the results.

The formation of a precipitation ring by staphyloenterotoxin in agar gel diffusion tests was studied by the modified method of Surgalla *et al.* (1954). The staphylotoxin was prepared from one of the enterotoxic strains (No. 127) isolated from food poisoning in Thailand. This strain was coagulase and gelatinase positive, fermented mannitol and grew in the presence of 7·5 per cent sodium chloride. Not less than 8 embryos died in 9 days when at least 10 organisms were inoculated into the chorioallantoic cavity of ten 10-day-old eggs (test of McCabe, 1962).

Growth in the medium of Favorite and Hammon (1941) at pH 7·4 was harvested after 36 hours, centrifuged at 3,000 r.p.m. for 15 minutes, dialysed against distilled water at 2 to 4°C. and evaporated in an air current. This was the crude toxin. The crude toxin was precipitated at −20°C. with methyl alcohol according to Casman (1958) and then purified by silica gel chromatography following the procedure of Bergdoll *et al.* (1952). Although this method gave good yields, its modification by Casman (1960) with ammonium sulphate saturated to 85 per cent and treatment with 20 per cent hydrochloric acid yielded more concentrated enterotoxin in later studies.

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All concentrates caused vomiting in Macacus monkeys when injected in amounts between 0·05 and 0·2 ml. kg of body weight. The toxic substances remained active for at least one month when preserved at −20°C. The crude toxin was tested against the human sera using the agar gel diffusion method on microscopic slides. The wells were 2 mm in diameter and 2 mm apart. The central well contained the crude toxin while the 4 outer wells were filled with sera to be tested. The Preer test (1956) was employed with all sera which gave at least one precipitation line when examined by the slide method. In the Preer procedure 0·1 ml. of the serum to be tested was put into a capillary of 2 mm. diameter. Five tenths of a per cent of Ionagar (Oxoid Co.) in the same buffer as used in the anti-α toxin determinations, pH 7·4, with 1 : 5,000 methiolate, was employed. The serum in the capillaries was carefully overlayed with 0·2 ml. of this agar, then with 0·1 ml. of the crude or purified enterotoxin that was adjusted to contain 1/100th of the amount per kg. of body weight necessary to produce vomiting in monkeys after intravenous injection. The formation of bands and their movements were read after 1, 7, 14 and 21 days and the antibody content was calculated according to Preer's formula (1956).

Agar gel diffusion experiments were carried out with the same sera also using 0·05 and 0·5 units of α, β and δ toxin as antigens. All tests were made in duplicate.

TABLE I

STAPHYLOCOCCAL ANTIBODIES IN RESIDENT THAIS AND UNITED STATES VISITORS IN THAILAND

Serum from	*No. Examined*	*α-antitoxin U*			*Anti-enterotoxin U*			*Against crude enterotoxin Total No. of mobile bands in Preer test*						
		1	*1–2*	*2*	*1*	*1–3*	*3*	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Healthy men															
Thai	100	16	54	30	72	17	1	3	4	14	38	29	10	1	1
U.S.	100	88	12	0	94	2	0	66	12	8	7	4	2	0	1
Staph. food poisoning															
Thai	21	2	3	16	0	10	11	0	0	2	9	7	2	1	0
U.S.	25	2	2	21	1	10	14	0	0	4	10	8	2	1	0
Staph. skin lesions															
Thai	100	2	2	96	82	16	2	0	1	2	43	30	14	6	2
U.S.	20	0	2	18	18	2	0	0	1	2	10	6	0	1	0
Diarrhoea of unknown origin															
Thai	42	9	13	20	19	18	5	1	0	8	14	10	5	2	2
U.S.	42	15	6	11	31	9	2	4	3	7	16	10	2	1	1
Cholera patients															
Thai	200	32	107	49	180	19	1	5	7	20	87	54	23	3	1
Cholera Convalescents															
Thai	100	10	53	34	87	12	1	2	2	16	36	31	8	2	1
Salmonellosis, shigellosis															
Thai	42	11	16	13	34	8	0	2	2	7	10	16	3	2	0
Trachoma															
Thai	16	8	6	2	13	2	1	2	2	3	3	2	1	2	1

Results and Discussion

The results of the experiments are shown in Table I. Since Ipsen (1940), Kientz and Walter (1964) and others (reviewed by Elek, 1959) observed that the sera of many "normal" persons contain α or δ antitoxin, it was believed that the suggestion of Kientz and Kümmel (1960) should be considered, *i.e.* that sera containing less than 2 units of anti-α toxin should be regarded as "normal." In this communication only the results with α hemolysin have been included since β antitoxin was seldom found. This may be due to the scarcity of this toxin in human-pathogenic staphylococci (Elek 1959). Measurable levels of δ antitoxin were even less frequent.

The difficulties encountered by Surgalla *et al.* (1954) and Casman (1958, 1960) in the evaluation of precipitin lines in agar gel diffusion tests with staphylococcal antigens were encountered also in these experiments. There were numerous precipitation lines. Many of them could not be identified. Table I shows that numerous sera formed more than three precipitation rings. None to seven bands were seen in agar gel diffusion tests with crude culture supernates. One of the bands which was neither identical with those formed by the α, β or δ toxin-antitoxin reactions when compared with the results obtained by using purified toxins, was considered characteristic for the enterotoxin. Its movement in the Preer tube permitted the estimation of the antibody content according to Preer's formula (1956). Using purified enterotoxin, this precipitation ring appeared as a single band, but in 27 per cent of the tests it split into two, and in 18 per cent into three, lines. This may be due to impure antigens or to several antibody-antigen systems present within the enterotoxin complex.

The anti-α antibody contents calculated from the results of the Preer tests were well in agreement with those achieved by titration according to Kienitz and Kümmel.

When the results in apparently healthy Thai and American personnel were compared, 30 per cent of the former had more than two U anti-α toxin in their sera while all recent arrivals from the United States had less than two U. The serum of 18 per cent of these Thais contained more than one arbitrary anti-enterotoxin units, while only two per cent of the Americans harbored more than one unit. The number of precipitation bands between sera and crude staphylococcal antigen followed a distributional curve with a maximum of three bands in Thai persons, while the number of bands steadily decreased in American newcomers. These findings point to a more extensive experience of the permanent Thai population to infection with staphylococci and probably reflects a higher degree of immunity to staphylotoxins.

Only few persons with proven staphylococcal food poisoning could be observed. Nevertheless, the findings presented in Table I show that when such conditions developed, the responses were identical both in resident Thais and in newly arrived Americans. High anti-α toxin values were recorded. The highest titre was 8 U, observed in an American. Except in one case, the anti-enterotoxin values were higher than 1 U. At least two precipitation bands were seen in these patients.

Compared with findings in healthy persons, staphylococcal skin lesions caused a considerable increase in the anti-α toxin levels but not in the anti-enterotoxin values.

The group with "diarrhoea of unknown origin" probably included a number of patients with unrecognised staphyloenterotoxicosis causing elevated antistaphylotoxin and anti-α toxin titres. Cholera, salmonellosis and shigellosis did not alter significantly the distribution of the antistaphylotoxin values. The number of patients with trachoma was too low to permit a statistical evaluation of the data obtained by testing their sera.

The α toxin of the staphylococci does not play a role in food poisoning. Elevated anti-α toxin values may be due to other staphylococcal infections, especially to those of the skin. The anti-α toxin titres, however, were always increased in staphylococcal food poisoning. Since the determination of anti-enterotoxin is difficult because of the preparation of proper standards and the evaluation of the results of the agar gel diffusion tests, anti-α toxin determinations with the aid of a simple hemolysin titration technique may be helpful in the diagnosis of staphylococcal food poisoning.

Casman (1960) called attention to the lack of cross-reactions among some enterotoxic *Staphylococcus* strains. The results of this study, therefore, pose two questions: was the strain used by us yielding low titres with the sera of new arrivals and high antitoxin levels in the local population for that reason, or are persons in countries with highly developed health standards generally lacking antistaphyloenterotoxin because of their infrequent exposure to such noxae?

The lack of sufficient amounts of sera did not permit parallel studies with great numbers of staphylococci. As an alternative, a comparative

study of enterotoxins purified according to Casman (1960) and using rabbit immune sera prepared against such enterotoxins of five additional Thai and 10 American strains were tested by the Casman modification of the agar gel diffusion method. Four of the Thai and eight of the American enterotoxins gave lines of identity in this test, proving that while the strain employed in the experiments described in this communication does not produce an antigen that could be used for the determination of antienterotoxin in all instances, the results are significant.

It may be added that the identity or the close relationship of the enterotoxins was independent of the phage susceptibility of the staphylococci. This has been reported previously (Elek 1959).

This study did not prove conclusively that the less frequent appearance of food poisoning in the Thai population is due to higher antistaphylococcal antibody levels in the serum but the results presented in this communication suggest that this may be the case.

There are numerous causes of diarrhoea and vomiting among visitors to tropical countries. Staphylococci are one of them. Streptococci have not been studied sufficiently as yet from this point of view.

The examinations of Eveland *et al.* (1954, 1954a) showed that the "tourist diarrhoea" in the Far East may be due to an assortment of gram-negative enteropathogenic micro-organisms, from *E. coli* to *Shigella*, as well as to physico-chemical factors, as eating highly spiced food, or dishes to which the traveller is not accustomed; by consuming drinks to which he or she is not used; and, perhaps, also other factors. Thus, even if the finding of relatively low antistaphylotoxin levels in some newcomers to the Far East might be construed as a reason for visitors developing staphylococcal food poisoning more readily than the local population, this should not be considered as the all-embracing answer to the problem of the "tourist diarrhoea" or the "diarrhoea of newcomers."

Summary

A sample of adult Thai permanent residents of Thailand had higher anti-α toxin and anti-enterotoxin serum levels than newcomers from the United States of America. There were also significantly more persons in the former group whose sera formed several precipitation lines in the Preer test against crude staphylococcal supernates.

Staphylococcal food poisoning led to elevated serum anti-α and enterotoxin levels.

Serum anti-α toxin values were increased both in staphylococcal skin lesions and in staphylococcal food poisoning.

Serum antienterotoxin levels did not increase significantly in staphylococcal skin infections, cholera, salmonellosis or shigellosis.

It is suggested that the anti-α toxin titration be employed as an aid in the diagnosis of staphylococcal food poisoning in newcomers to the tropics if other staphylococcal infections can be excluded.

"Tourist diarrhoea" may be due to many agents. Staphylococci are only one of the numerous causes of that disease.

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